REMARKS

Claims 1, 2, 4-9, 13-27, 29-43, 45, and 46 were pending prior to this response. By the present communication, the first paragraph of the Specification has been amended to correct an inadvertent error in the filing date of the parent application in response to a note in the Filing Receipt that the filing date in the application did not accord with PTO records. In the claims, new claims 47-49 have been added, claims 1, 2, 4-9, 13-16, 18, 20-23, 26-28, 33, and 35-38 have been cancelled without prejudice, and claims 17, 19, 24, 25, 29, 30, 31, 32, 34, 39, 40, and 43 have been amended to define Applicants' invention with greater particularity. The amendments add no new matter, being fully supported by the Specification and originally filed claims. Support for new claim 49 is found on page 32 of the Specification. Accordingly, claims 17, 19, 24, 25, 29, 30-32, 34, 39-43, and 45-49 are currently pending.

The references cited at the end of this Response were all submitted with the [un-entered] Response to the Final Rejection filed herein on March 18, 2005. To avoid unnecessary build-up of paper in the official files for this application, the cited references are not being submitted again as attachments to this Response. The Examiner is directed to refer to the cited references as already of record in this application. If, for any reason, the cited references cannot be found for examination of this Response, Applicants request the Examiner to call their representative, whose name and telephone number appear at the end of this Response.

The Advisory Action re New Matter

The Advisory Action (continuation sheet) asserts that addition of new claims 47-48 would introduce the new issue of culturing early attaching cells to produce one or more unspecified agents, prior to direct injection into muscle tissue. In addition, the Examiner asserts that, as examined, a culturing step was directed to produce early attaching cells rather than culturing to produce "unspecified agents". In new claim 47 presented by the present communication, which depends from claim 17, the expressed agents are not "unspecified", but are one or more of the agents named

in claim 17. In addition, Applicants would remind the Examiner that in claim 17 as previously examined, it was a "growing" step, not a "culturing" step in which the early attaching cells were produced. New claim 47 reflects the subject matter of the previously examined claims by reciting a culturing step prior to the injecting step. In the culturing step the transfected cells are allowed to express one or more of the agents encoded by the polynucleotides with which the early attaching cells have been transfected. Applicants submit that the subject matter of the claims as amended is fully supported by the specification and original claims.

The Attachments Allegedly Missing From the Previous Response

The Final Office Action alleges that there were no copies or attachments describing Applicants' post-filing studies submitted as attachments with the prior Response filed on September 23, 2004. However, the postcard mailed with the Response shows that the Response was 27 pages including attachments A-C. Thus, the references attached to the Response must have been mislaid by the PTO upon their receipt. For the convenience of the Examiner, Applicants supplied additional copies of the attachments allegedly missing from the Response filed on September 23, 2004, with the Response to the Final Office Action filed herein on March 18, 2005. Applicants request the Examiner's consideration thereof in light of the argument in the prior and the present Responses.

The Rejection under 35 U.S.C. § 112, First Paragraph

Applicants respectfully traverse the rejection of claims 1, 2, 4-9, 13-27, 29-43 and 45-46 as allegedly lacking an enabling disclosure in the Specification. As claims 1, 2, 4-9, 13-16, 18, 20-23, 26-28, 33, and 35-38 have been cancelled without prejudice, the following remarks will pertain to the remaining claims as currently amended. All presently pending claims have been amended to pertain to injection of early attaching cells obtained from bone marrow that have been transfected with an adenoviral vector comprising a polynucleotide encoding one or more angiogenic agents selected from hypoxia inducing factor-1 (HIF-1), endothelial PAS domain protein 1 (EPAS1), Monocyte Chemoattractant Protein 1 (MCP-1), granulocyte-monocyte colony stimulatory factor (GM-CSF), PR39, a fibroblast growth factor (FGF), and a nitric oxide synthase (NOS). The Examiner acknowledges that the specification is enabling for treatment of hind-limb ischemia in mice, but alleges that that the Specification does not reasonably provide enablement for promoting angiogenesis in "any tissue/organ of any animal or heart/limb tissue in a human" (Final Office Action, page 2).

According to the Final Office Action, the test for enablement is whether one skilled in the art can make and use the claimed invention based on the description in the specification coupled with information known in the art without undue experimentation (Final Office Action, page 2). The standard of undue experimentation is that set forth in In re Wands, including the following

factors: 1) scope/breadth of claims; 2) nature of the invention; 3) state of the art and predictability; 4) amount of guidance provided; 5) number of working examples; and 6: amount of experimentation required (In re Wands, 8 USPQ 2d 1400 (Fed. Cir. 1988). The factors will be addressed in the order they were presented in the Office Action.

Scope and breadth of claims. The Advisory Action states that a proposed amendment of the claims to cells that "contain an adenoviral vector comprising [genes encoding various angiogenic factors]" would read on cells "inherently containing" said adenoviral vectors (Advisory Action, Continuation sheet). However, to prevent such an unintended interpretation of the claims at issue, the currently proposed amendment to the claims requires instead that the cells "have been transfected" with such an adenoviral vector.

In the Final Office Action, the Examiner asserts that the claims are broader than the scope of enablement because the composition claims are limited as "therapeutic" and are directed to transfected early attaching cells and the method claims are directed to enhancing collateral blood vessel formation in "patients", thus indicating that the claims are directed to "gene therapy" in humans. In addition, the Examiner asserts that the ABMs are transfected with nucleic acids obtained from "any source" to express the named factors with the expectation of enhanced angiogenesis.

However, Applicants submit that the terms "therapeutic" in the composition claims and "patient" in the method claims are not necessarily a delimiter of human therapy. It is quite clear from the description of the invention that therapy can be performed in animals as well as humans. By the same token, Applicants submit that the proposed deletion of the term "therapeutic" from the composition claims would not "delimit such cells as no longer being therapeutic" as asserted by the Examiner in the Advisory Action (Advisory Action, continuation page). Applicants submit that the claimed composition has inherent therapeutic properties when used according to the teachings of the Specification by virtue of the inherent properties of the composition as claimed, whether or not the term "therapeutic" is contained in the preamble to the claim. Thus, the claim scope would not be affected by removal of the term "therapeutic" from the claim preamble.

In view of the Examiners comments regarding the ambiguous implications of the terms "therapeutic" and "patients" for claim scope (as set forth in the Final Office Action and the Advisory Action), Applicants wish to make absolutely clear in the record that the claim scope is not interpreted to be limited to use in humans. The term "therapeutic" as used in the composition claims is a reflection of inherent properties of the claimed composition and bears no indication that the claims are limited in scope to use in humans. The term "patient" has been deleted from the method claims to traverse the Examiner's assertion that use of the term "patient" *requires* a human subject. This amendment is made in the light of the Examiner's comments, in effect, to ensure that the claims are examined as suitable for use in animals as well as humans, in other words, to ensure full claim scope, not to the narrow the claim scope.

The application describes use of the invention methods and compositions in mice and the methods described therein have been used by others in such animals as pigs. As discussed below in connection with various studies in the art performed since the filing of the application, the teachings of the Specification have been corroborated in various human subjects as well. In addition, the tissue recited in the method claims as presently amended is "heart or limb muscle tissue" suffering from restricted blood supply, language that pertains to both human and animal subjects. Thus, neither the composition nor the method claims, as presently amended, recite language that can be construed to *require* a human subject.

The Examiner's concern in the Final Office Action regarding the unspecified "source" of the nucleic acids used to transfect the cells is not clear. Applicants submit that the polynucleotides used to transfect the bone marrow derived cells in the invention compositions and methods can be non-autologous or non species-matched nucleic acids. Applicants respectfully submit that the cytokines and other factors recited by the claims are transcription factors and other very small proteins, and/or proteins that are highly conserved across species. Moreover, the nucleic acid sequences encoding the named agents were known at the filing of the application, including those for both murine and human agents. Although the Specification does not address the source of the polynucleotides or teach the use of species-matched polynucleotides, those of

skill in the art practicing the invention at its filing date would have considered it routine best practice to use a species-matched DNA for encoding the desired cytokine or angiogenic factor.

Further, with regard to enablement, the Examiner has provided no evidence in support of the implied conclusion that use in vivo of a non-homologous or non-species matched agent would either fail to be effective or would generate a harmful immune response in the subject. Applicants submit that when the proteinaceous molecule expressed is highly conserved across species and/or as small as HIF-1, EPAS1, MCP-1, GM-CSF, PR39, FGF or NOS the source of the nucleic acid does not determine the effectiveness of the encoded molecule, even in humans. Applicants invite the Examiner to provide any proof of harmful immune response in humans to such cytokines or factors expressed from non-human polypeptides. Lacking such evidence, Applicants submit that the scope and breadth of the currently amended claims are not broader than the disclosure and that the claims can be practiced by those of skill in the art without undue experimentation.

Nature of the invention. The Office Action asserts that all claims are directed to human treatment due to use of the term "patient" in the method claims and "therapeutic" in composition claims. Applicant disagrees that these terms identified by the Examiner necessarily require or refer to a human subject for the reasons discussed above. However, for clarity the method claims, as defined by amended claim 17, have been amended to delete any language that could be construed to require treatment of a human subject. Applicants respectfully submit that the invention composition comprising bone marrow-derived early attaching cells transfected with an adenovirus vector comprising polynucleotide(s) encoding at least one of the recited agents is intended to be and can be used in animal model research and in animal and human therapy and is presently being so used by many experimenters, as is discussed in full detail below.

In addition, it should be noted that the Examiner does not allege that the Specification fails to teach those of skill in the art how to make the claimed composition or how to use it to enhance collateral blood vessel formation by injection into muscle tissue, as defined by amended claim 17. Applicants submit that the Specification contains Examples that describe the procedure used to make and use the claimed composition in such applications. Example 7, for instance, describes harvesting of bone marrow cells from pigs, culture of the cells in vitro to obtain the "early

attaching cells," which are characterized as "mostly monocytes, endothelial precursor cells, or other hemopoietic lineage cells" (¶ [0108]), in other words progenitor cells. Similarly, the procedure for transfection of the early attaching cells using an adenovirus vector is fully described (¶[0110]. In vivo use of early attaching cells that have been transfected with a polypeptide encoding HIF-1α in a mouse ischemic hind limb model showed that transduction of MSCs with HIF-1α was effective to "optimize the collateral-enchancing effects of a cell-based strategy for increasing collateral flow in ischemic tissue" (¶ [0125] when the cells were injected into hind limb muscle tissue suffering a loss of blood supply.

Applicants submit that the making and use of the invention composition in the invention methods, as defined by the currently amended claims, is described in the Specification in such a way as to enable those of skill in the art to practice the invention without undue experimentation.

State of the art/Unpredictability of the art. The Final Office Action asserts that the Specification does not teach how to use the claimed methods and compositions therapeutically commensurate with the scope of the claims. In particular, the Examiner asserts that at the time the application was filed, gene therapy was a "highly unpredictable art with poor efficiency of delivery of the transgene to the target cells, poor transformation efficiency of target cells, unpredictable and transient expression of the transgene in target cells, etc." (Office Action, page 4).

At the outset it should be noted that all currently amended claims, both composition and method, require steps that distinguish the invention from the types of methodologies described in the articles cited by the Examiner as showing unpredictability in the art of "gene therapy." In one embodiment of the invention method, as defined by amended claim 17, autologous bone marrow derived progenitor cells are transfected ex vivo and the transfected *donor cells are used as a vector* to deliver the transgene into the subject's muscle by injection. Thus the adenoviral vector is sequestered in the donor's own bone marrow-derived cells to shield the vector from detection by the immune system of the recipient, considerably lessening, if not completely eliminating, the

type of recipient immune response to the viral vector described by the St. George reference of record herein as cited by the Examiner.

Alternatively, in the invention methods the transfected donor cells are further prepared by ex vivo culture to obtain conditioned medium containing not only the endogenous products secreted by the cells but also protein product of the transgene and/or the protein products derived from the effects of the transfected gene product on the transduction pathways of the transfected cell. At least some of the transgene's expression products will be obtained ex vivo in the conditioned medium, *prior to injection of the conditioned medium* into target ischemic muscle tissue. Thus in the case wherein the composition is the conditioned medium produced by the subject's transfected cells, the angiogenic agents in the conditioned medium are of two types, those endogenous to the subject's cells and at least some expressed from the transgene before the invention composition is injected into the muscle tissue.

Further in support of the rejection for lack of enablement, the Examiner states: "More particularly, with respect to gene therapy, the transduced cells in a human patient would need to express the angiogenic factor for a threshold period of time to promote angiogenesis..." (Final Office Action, page 4). The application teaches that cellular survival of the transgene and gene product expression in situ following injection of transduced cells into adductor muscle of mice was maintained at least through day 14. Studies conducted by the inventors and others have shown that adenoviral gene expression lasts for at least weeks, and perhaps months. The physiological time table for increasing collateral flow in ischemic muscle tissue is much briefer than 14 days. In fact, Applicants have demonstrated in data published using normal dogs that administration of FGF-2 to ischemic dog hearts for only 2 days increases collateral flow. In many cases the collateral vessels are in incipient form and rapidly undergo enhancement to support increased blood flow. Thus, the physiological time table for development of collateral blood vessels in humans and in animals is well within the time that transduced cells, as required by the present claims, can be expected to express the transgene to promote development of collateral blood vessels.

In addition, Applicants submit that the specification teaches in great detail the research that underpins the determination that HIF-1 activates human cells to pump out agents that enhance collateral blood vessel development under hypoxic conditions. Applicants' studies have shown that expression of a transgene, such as HIF-1α, in vitro *during culture of the transfected cells* has the effect of activating the bone marrow-derived progenitor cells for inducing collateral vessel enhancement even more than subjection to hypoxia, the body's (human and animals) endogenous mechanism for enhancing collateral blood vessel development in muscle adjacent to ischemic tissue.

Applicants submit that the Examiner has provided no reason in support of the conclusion that a substantially different result than would be obtained in humans in vivo. On the other hand, Applicants and others have shown in published studies that development of collateral blood flow in muscle tissue upon injection of the transduced blood marrow progenitor cells takes place rapidly following onset of ischemia (as quickly as 3 days after ischemia onset). Thus, there is no support for the Examiner's inference that the transgene would not be expressed for a sufficient time to effect enhanced development of collateral blood vessels in human muscle tissue into which the transfected cells are directly injected.

Moreover, the Examiner's concerns regarding supposed lack of efficiency of transfection of the transgene in target cells are unfounded. Applicants have shown in the Specification that at least in the case of early attaching cells obtained from bone marrow, these cells are transfected with a heterologous gene ex vivo, as is required in the claimed invention, at efficiency of "over 90%" (Specification, ¶ [0117]). High transfection efficiency in *human* progenitor cells has also been shown (See attached Exhibit A, T Watanabe et al., Blood (1996) Jun 15; 87(12):5032-9). While the Examiner's assertions regarding inefficiency of transfection and expression of transgenes may be valid as applied to conventional routes of gene therapy, the facts show that the transfection efficiency of bone marrow early attaching cells (i.e., progenitor cells) is extremely high.

The Examiner has also asserted that deleterious viral proteins will be produced in the conditioned medium resulting from use of an adenovirus vector" (Office Action, page 4) in the

invention compositions and methods. The facts show that this fear is equally unfounded. Adenoviral vectors, as the term is used in the art, are not intact adenoviruses, but are routinely constructed so that essentially no viral genes are transcribed. The only gene transcribed is the transgene that has been inserted into the vector. For example, the Specification describes such "replication-deficient adenoviral vectors" in ¶ [0050-51]. The actual adenoviral HIF-1α/VP16 vector used to transfect MSCs in Example 7 of the Specification is covered by U.S. Patent No. 6,093,567 and was used in collaboration with Genzyme Corporation, owners of the '567 patent. The same construct is presently being injected directly (without ex vivo transfection of bone marrow progenitor cells) into *human* heart and limb muscle in FDA approved clinical trials being conducted by Genzyme Corporation to show efficacy for angiogenesis. Thus, it appears that the FDA believes that the use of adenoviral vectors is sufficiently safe and effective for use in humans as well as in other mammals.

The St. George reference cited by the Examiner as proof of the unavoidable dangers of human gene therapy actually supports Applicants statements above with regard to local administration of adenoviral vectors as follows:

> The January 2002 issue of Human Gene Therapy contains two studies describing the responses in nonhuman primates. Studies in non-human primates may be the most instructive to our understanding of Ad-mediated toxicity as the responses have been shown to mirror those described in patients. The findings of studies conducted in primates, including humans, are summarized briefly in Table 1. It is important to note that on balance, other manuscripts in that issue of Human Gene Therapy concluded that in studies using several routes of local administration of low to intermediate doses of *Ad vectors were well-tolerated in humans*.

(St. George, page 1137, first col; emphasis added.) Thus, the very art cited by he Examiner as proving that adenoviral vector are too dangerous for use in humans has shown that adenoviral vectors are on balance safe and effective in humans as in primates.

Applicants respectfully disagree as well with the Examiner's assertion that insertional mutagenesis is a dreaded potential complication of adenoviral gene delivery, including the development of leukemia. It is well known in the art that *retrovirus* can insert into the genome and thereby can cause insertional mutagenesis. Retrovirus have recently been shown capable of

causing leukemia. However, it is also very well known in the art that adenovirus vectors of the type described in the Specification do not insert into the genome. Adenoviruses always reside in an extrachromosomal location. Applicants submit that adenoviruses have never been found to cause insertional mutagenesis and never have been associated with cancers, whether injected directly into muscle or not.

The adenovirus vector encoding HIF-1/VP16 used in the mouse model described in Example 7 was provided by Genzyme Corporation under the terms of a Research Agreement. Attached is a printout of a news release from the University of Pittsburgh Medical Center evidencing that the Genzyme vector has been approved by the FDA for use in human clinical trials being conducted by Genzyme Corporation. According to the news release, similar adenoviral vectors have been used in seven gene therapy clinical trials, with three more in progress. Thus, the Examiner's concerns that use of an adenovirus vector in the invention methods and composition might result in harm to a human patient, sufficient to make use of the invention unsafe are not shared by the FDA, at least not to the extend that human trials have been banned.

The one recent adverse incident involving an adenoviral vector when used for human gene therapy (in a clinical trial conducted by the University of Pennsylvania) should be distinguished from the present invention. The University of Pennsylvania clinical trial involved a case wherein the adenoviral vector was injected in very high concentrations (10^{13}) directly into the artery supplying the patient's liver. Thus method of employing an adenoviral vector in the University of Pennsylvania study lacked the precaution that the vector was sequestered in the patient's own progenitor cells that had been transfected ex vivo with the adenoviral vector.

Applicants have shown in Example 6-8 of the Specification that bone marrow-derived early attaching cells, such as MSCs, can be transfected with an adenovirus vector with high efficiency and that conditioned medium alone obtained by growing such transfected cells contains numerous angiogeneic cytokines and exerts biologic effects that are compatible with the capacity of the injected muscle tissue to develop collaterals. Thus, Applicants studies have shown a very high transfection efficiency of the early attaching cells and transient transgene expression of

sufficient duration to accomplish the claimed result of enhanced collateral development of blood vessels in the injected muscle tissue. This study as well as the arguments above effectively traverse the Examiner's arguments that use of an adenoviral vector to transfect cells used in cell therapy is unpredictable and unreliable.

Amount of guidance provided/Number of working examples The Examiner's assertions with regard to these Wands categories can be summed up in the following statement: "...no significant relevant guidance is provided with respect to gene therapy or transfection of ABMs and treatment of a human patient" (Office Action, page 7). However, Applicants submit that the studies described in Example 6-8 of the Specification are sufficient to guide one of ordinary skill in the art to perform the claimed invention in humans without undue experimentation, as shown by published studies performed by others since the publication of the present invention. For example, Exhibit B formerly submitted in this application (E. C. Perin et al., *Circulation* (2003) 107:r75-r83) describes how transendocardial injection of mononuclear cells obtained from autologous bone marrow cells into patients with end stage ischemic heart disease safely promoted neovascularization and improved perfusion and myocardial contractility, both of which depend upon collateral vessel development. This study, which discusses the similarity of outcomes in animal and human studies, shows that even without transfection of the cells with an adenoviral vector expressing an angiogenic factor, humans react to intracardiac injection of bone marrow-derived progenitor cells in the same way that Applicants illustrated in the mouse hind-limb study. A similar therapeutic result is described in formerly submitted Exhibit C (M.B. Britten, et al., *Circulation* (2003) 108:2212-2218), resulting from infusion of untransfected bone marrow-derived progenitor cells into infarcted myocardial artery of human subjects. MRI was used in the Britten study to show significant improvement in global LV EF and reduced end-systolic volumes achieved using this method, which again indicates development of collateral blood vessels in the treated human patients.

Yet another study in humans is described in formerly submitted Exhibit D (Extract of K.C. Wollert et al., *Lancet* (2004) 364(9429):121-2), which describes tests in 60 human patients

in which intracoronary transfer of autologous bone marrow cells promoted improvement of left-ventricular systolic function in patients after acute myocardial infarction.

Taken together, these studies support the teachings of the invention that injection of autologous bone marrow cells, especially bone marrow-derived early attaching cells that secrete at least one of the transgenic angiogenic agents known in the art to activate production by the cells of endogenous angiogenic agents as claimed herein, is effective in humans, as in animals, to enhance collateral development, even in subjects after acute myocardial infarction.

Further, in In re Brana, *in vivo* human data was not necessary because there was reasonable basis for assuming the efficacy of methods in the mouse model was sufficient to meet enablement requirements. In re Brana 51 F. 3d 1560, 34 USPQ 2d 1436 (Fed. Cir. 1995). Similarly in the claimed invention, there is a reasonable basis for assuming that the method of injecting into heart or limb muscle bone marrow-derived early attaching cells that have been transfected with an adenoviral vector containing a polynucleotide encoding at least one of the recited angiogenic factors, or conditioned medium produced by culturing such transfected cells in the mouse model are sufficient to meet enablement requirements. Hence, in vivo human data is not necessary in order to meet the enablement requirement. Therefore, as in In re Brana, those of ordinary skill in the art can assume that the methods and disclosures of the claimed invention would be operable for their intended purpose when used in humans.

Number of working examples. The Final Office Action states that the Specification lacks examples commensurate in scope with the claims. With regard to lack of a working example for method(s) of injecting transfected bone marrow-derived early attaching cells into heart muscle in animals, or heart or limb muscle tissue in humans, the ruling in In re Brana supports the present claimed invention in that sufficient description in the specification and animal model methods are provided that an in vivo human example is not necessary to meet the enablement requirement, as discussed above.

Further, the training materials for examining patent applications states that "[t]he presence of only one working example should never be the sole reason for making a scope rejection, even though it is a factor to be considered along with all the other factors. To make a

valid rejection, one must evaluate all the facts in evidence and state why one would not expect to be able to extrapolate that one example across the entire scope of the claims." Training Materials for Examining Patent Applications with Respect to 35 U.S.C. §112, First Paragraph-Enablement Chemical/Biotechnical Applications, reprinted in Iver P. Cooper, *Biotechnology Law*, App. H-156, App. H-177 (2000). Therefore the Final Office Action must state exactly why the existing examples cannot be expected to be extrapolated by those of skill in the art across the entire scope of the claims in view of the knowledge in the art. Applicants submit that the burden of proof has not been met in the Final Office Action.

Thus, although the level of skill in the art required to perform the claimed invention is high, Applicants respectfully submit that there are sufficient examples and guidance in the specification for those of skill in the art to practice the invention as defined by the current claims without undue experimentation. Accordingly, reconsideration and withdrawal of the rejection for alleged lack of enablement are respectfully requested.

In view of the above amendments and remarks, Applicants request favorable action on all pending claims. If the Examiner would like to discuss any of the issues raised in the Office Action, the Examiner is encouraged to call June Learn at (702) 614-7219 so that a prompt disposition of this application can be achieved.

Respectfully submitted,

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